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REPRODUCTION OF *ANOPLODIUM HYMANAE*, A TURBELLARIAN FLATWORM (NEORHABDOCOELA, UMAGILLIDAE) INHABITING THE COELOM OF SEA CUCUMBERS; PRODUCTION OF EGG CAPSULES, AND ESCAPE OF INFECTIVE STAGES *WITHOUT* EVISCERATION OF THE HOST

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ABSTRACT

Anoplodium hymanae, a member of the turbellarian family Umagillidae, parasitizes the holothuroid *Stichopus californicus* along the northeastern Pacific coast. As in several other species of *Anoplodium*, egg capsules are released into the perivisceral coelom of the host. The egg capsules of *A. hymanae* become ensheathed by host coelomocytes and are then accumulated in masses that are usually about 1 mm in diameter. A single mass from a host that is moderately infested by *Anoplodium* may contain up to several hundred egg capsules. The masses pass out of the host on a daily basis, presumably through previously undescribed ducts that connect the coelom to the lumen of the posterior end of the rectum. Some masses of coelomocytes with egg capsules may be released if the host eviscerates but, contrary to previous hypotheses, evisceration is not required for completion of the life cycle.

Anoplodium hymanae continues to grow after attaining reproductive maturity. The size of the egg capsules, and thus of the larvae, varies with the size of the parent worm.

INTRODUCTION

The turbellarian family Umagillidae constitutes one of the principal groups of metazoan endoparasites of echinoderms and sipunculans (Hyman, 1955, 1959; Barel and Kramers, 1977). The family belongs to the suborder Dalyellioidea within the order Neorhabdocoelida (Crezée, 1982)—the suborder of Turbellaria that is often hypothesized to have the closest common ancestry with the Monogenea, Digenea, and Cestoda (Bresslau and Reisinger, 1928; Karling, 1970; Brooks *et al.*, 1985; Ehlers, in press).

Approximately 50 species of umagillid flatworms have been described (Cannon, 1982; Komschlies and Vande Vusse, 1980a, b; Shinn, 1983a; Westervelt, 1981; and references therein; see also Kawakatsu, 1983), but the life history has been elucidated for only one species (Shinn, 1983b). That worm, *Syndisyrinx franciscanus*, inhabits the intestine of certain sea urchins (Lehman, 1946). Egg capsules of *S. franciscanus* are released into the intestine of the host and pass out of the host with the feces. Embryogenesis occurs in the sea. Fully developed embryos remain in the egg capsules in a dormant state until the capsules are ingested by a sea urchin. The embryos are induced to hatch by some component of the sea urchin intestinal fluids (Shinn, 1983b).

Eleven species of umagillid flatworms are reported to inhabit the perivisceral coelom of aspidochirote holothuroids. Nine of these belong to the genus *Anoplodium* and, as

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far as is known, live exclusively in the coelom. The other species, *Macrogynium ovalis* and *Umagilla forskalensis*, are reported to live in both the coelom and intestine (Snyder, 1980; Cannon, 1982; and references therein). The reproductive biology of coelom-inhabiting umagillids must be more complex than that of intestine-inhabiting species because mechanisms must exist for infective stages of the former to escape from the body cavity of one host and enter the body cavity of a new host.

Several species of umagillid flatworms that inhabit the coelom of holothuroids deposit their egg capsules directly into the coelom (*Anoplodium parasita:* Schneider, 1858; Changeux, 1961; *A. stichopi:* Jespersen and Lützen, 1971; and an unspecified species: Arvy, 1957). The capsules accumulate in masses of host coelomocytes, commonly known as brown bodies. Schneider (1858) observed that coelomic egg capsules of *A. parasita* sometimes contain fully developed embryos but that coelomic capsules are never hatched. He inferred that the capsules pass out of the host before hatching. Changeux (1961) made similar observations on this species and suggested that the egg capsules are cast out when the hosts, *Holothuria tubulosa, H. polii,* and *H. stellata,* eviscerate (auto-expel the intestine and respiratory trees).

Jespersen and Lützen (1971) discovered that brown bodies containing egg capsules of the umagillid *Anoplodium stichopi* are, indeed, cast out of the coelom when its host, the holothuroid *Stichopus tremulus,* eviscerates. There is also evidence that spontaneous evisceration is involved in the release of infective stages of some species of the bizarre entoconchid gastropods and gregarine protozoans that inhabit holothuroids (Tikasingh, 1962; Lützen, 1979). These parasites develop in association with the host's viscera but typically protrude into the perivisceral coelom. As they mature, some species break their connection to the host organs and come to lie free in the coelom.

Many species of holothuroids have been observed to eviscerate in the laboratory, and are thought to eviscerate at least occasionally in the field (Bakus, 1973). In addition to *Stichopus tremulus,* these include *Holothuria forskali* (= *H. nigra*) which harbors the umagillid *Umagilla forskalensis* (Minchin, 1892; Westblad, 1953; Cannon, 1982), *H. tubulosa,* which harbors *Anoplodium parasita* (Schneider, 1858; Changeux, 1961), and *Stichopus californicus,* which harbors *A. hymanae* (Swan, 1961; Shinn, 1983a), as well as many species that are not reported to be parasitized by coelomic umagillids. For the species of *Holothuria,* evisceration is apparently only sporadic. In contrast, *Stichopus californicus* and at least a portion of some populations of *S. tremulus* have been thought to eviscerate spontaneously every autumn (Swan, 1961; Jespersen and Lützen, 1971, respectively). Lützen (1979) suggested that seasonal evisceration provides a dependable means of escape for infective stages of parasites infecting the coelom of at least some holothuroids.

Contrary to what one would expect if seasonal evisceration was the only, or most important, means of escape of infective stages from the coelom of *Stichopus californicus* or *S. tremulus,* maturation of their coelomic parasites, and formation of egg capsules or other infective stages by the parasites are not seasonally restricted (Lützen, 1979). Recently, Fankboner *et al.* (1981) reported that the viscera of *S. californicus* undergo a seasonal resorption and regeneration rather than evisceration. Similarly, *S. japonicus,* which harbors the umagillid *A. mediale,* resorbs the gut seasonally and is not known to eviscerate annually (Ozaki, 1932; Tanaka, 1958). While there is clearly a need for more information about the natural occurrence of evisceration, these observations suggest that means other than spontaneous evisceration exist for the passage of infective stages of coelom-inhabiting parasites from the host.

Kincaid (1964) hypothesized that entoconchid gastropods can induce evisceration of the host at times that are appropriate for release of infective stages. Lützen (1979) discounted this hypothesis because the host that Kincaid was studying, *Stichopus*

californicus, as well as *S. tremulus* which also harbors entoconchids, are virtually never found with regenerating viscera except during what has been thought to be the fall evisceration period. It has been suggested that infective stages are liberated when the hosts die, but Lützen (1968, 1979) mentioned that holothuroids are probably relatively long-lived and that infective stages are more likely to be eliminated during even occasional eviscerations of the hosts. Massin *et al.* (1978) suggested that gregarine protozoans that inhabit the deep sea holothuroid *Psychropotes longicauda* might use intermediate hosts, such as eulimid gastropods that suck the coelomic fluid out of holothuroids, to help them escape the coelom. There is, however, no evidence to support this interesting possibility. None of these hypotheses are as attractive as that of spontaneous evisceration, but there have been no attempts other than Lützen's (1979) to solve this problem in detail.

This paper describes certain aspects of the life history of *Anoplodium hymanae* Shinn. This umagillid inhabits the coelom of the aspidochirote holothuroid *Stichopus californicus* in the northeastern Pacific. The morphology of egg capsules, rate of production of egg capsules, duration of embryogenesis, and the importance of evisceration for release of infective stages are analyzed. In addition, I describe experiments which reveal that infective stages escape the coelom on a regular basis in the absence of evisceration of the host. A new morphological feature of stichopodid holothuroids, by which escape of infective stages may be accomplished, is described. The mechanism of invasion of new hosts, the morphology of larvae, and seasonal variations in the intensity of infestation are described in another paper (Shinn, 1985). These results have been summarized in an abstract (Shinn, 1983c).

Materials and Methods

Collection and dissection of hosts

Specimens of *Stichopus californicus* were collected by divers near Colin's Cove, San Juan Island, Washington (48°33′N, 123°00′W, 2–15 m deep). The sea cucumbers were all more than 20 cm long. They were kept in running sea water aquaria at the Friday Harbor Laboratories (FHL). Those used for censusing were dissected within a few days of collection.

Sea cucumbers were sliced open with a razor blade and the coelomic fluid was collected in a dish. The viscera and body wall were submerged in sea water in a second dish. The dishes were placed on black cloth so that the white worms would be more visible. Worms were transferred via a Pasteur pipette to a separate container of filtered sea water. Brown bodies (*i.e.*, the masses of coelomocytes that contain egg capsules) were collected from the viscera, the inner side of the hosts' body wall, and the bottom of the dishes. After the coelomocytes settled out of suspension in the dish of coelomic fluid (about 1 h), the fluid was decanted and the bottom of the dish was examined with a dissecting microscope for additional small worms and egg capsules.

Maintenance of animals and egg capsules

Flatworms and egg capsules were kept at low densities in filtered sea water. Short, wide-mouth jars were used as containers; they could be capped loosely in order to prevent evaporation. A large air space was left in the jars. The sea water was usually changed every two days. The jars, and 20 liter aquaria in which sea cucumbers were kept during experiments, were partially submerged in continuously flowing water in sea tables. The temperature ranged from 8–12°C depending on the time of year. Unless specified, the light regimen was not controlled; it consisted of alternating periods of subdued light (from windows) and dark.

To determine the rate of production of egg capsules, adult worms were dissected from sea cucumbers and kept in the dark. The worms appeared healthy for 3–5 days under these conditions. The numbers of egg capsules deposited by the worms during the first 48 h were used to calculate rates of capsule production. Capsules produced and released by these worms were kept in separate containers of filtered sea water. Embryogenesis was followed by periodically examining the intact capsules with a compound microscope.

Effects of evisceration

When recently collected holothuroids eviscerated in aquaria, expelled worms and brown bodies with egg capsules were collected and counted. The eviscerated holothuroids were isolated in separate aquaria for one day and then dissected. Worms and egg capsules remaining in the coelom were counted. Holothuroids were judged to have eviscerated in the field when the following criteria were met: (1) they did not eviscerate after collection, and (2) the digestive tract consisted only of the open-end rectum and a stub of the intestine attached to the aquapharyngeal bulb. Specimens that had a regenerating intestine were not categorized as having eviscerated because of the possibility of confusing specimens that had eviscerated with those that had resorbed the digestive tract (see Fankboner *et al.*, 1981).

Release of infective stages from the host

To determine if egg capsules or worms are voided from hosts by mechanisms other than evisceration, specimens of *Stichopus californicus* were put into individual 20 liter aquaria that had been fitted with a 0.5 cm mesh screen four cm above the bottom. This arrangement allowed objects coming out of the holothuroids to settle through the mesh, and thus prevented ingestion of these objects by the sea cucumbers. Sea water was allowed to trickle into the aquaria from above. The sea cucumbers were not fed. Every two days the water in the aquaria was filtered through a 143 μm Nitex screen and examined for brown bodies, egg capsules, and worms. At the end of the experiments (7–20 days), the animals were dissected. The worms and egg capsules in the coelom were counted. This procedure was repeated at different times of year using a total of eight *S. californicus*.

To locate coelomic ducts through which egg capsules of *Anoplodium hymanae* might escape the host, the perivisceral coelom of each of several large *Stichopus californicus* was injected with 10 ml of a 1% solution of methylene blue in sea water (technique of Anderson, 1966). The animals were held with the right side uppermost so that gravity would pull the internal organs away from the site of injection; this was located midway along the right side of the body. At various times over the next two days, the injected specimens were anesthetized in a 1:1 solution of 7.5% $MgCl_2$:sea water. They were then submerged in a shallow dish, and examined with the aid of a dissecting microscope. Pressure was applied to the body in order to force the dye out of any openings that might exist in the body wall.

To determine if egg capsules are transported out of the host by migration of egg capsule-laden masses of host coelomocytes through the body wall, or wall of the intestine, rectum, or respiratory trees, these parts of highly infested sea cucumbers were examined with the dissecting microscope. Dark masses of cells resembling brown bodies were teased apart with insect pins, or were excised, mounted on a slide, and examined with a compound microscope for the presence of egg capsules. Large pieces of the digestive tract and respiratory trees were also compressed between two slides, then examined with a compound microscope. In addition, the body wall of two *Stichopus californicus* that each contained more than 100 *Anoplodium hymanae* were

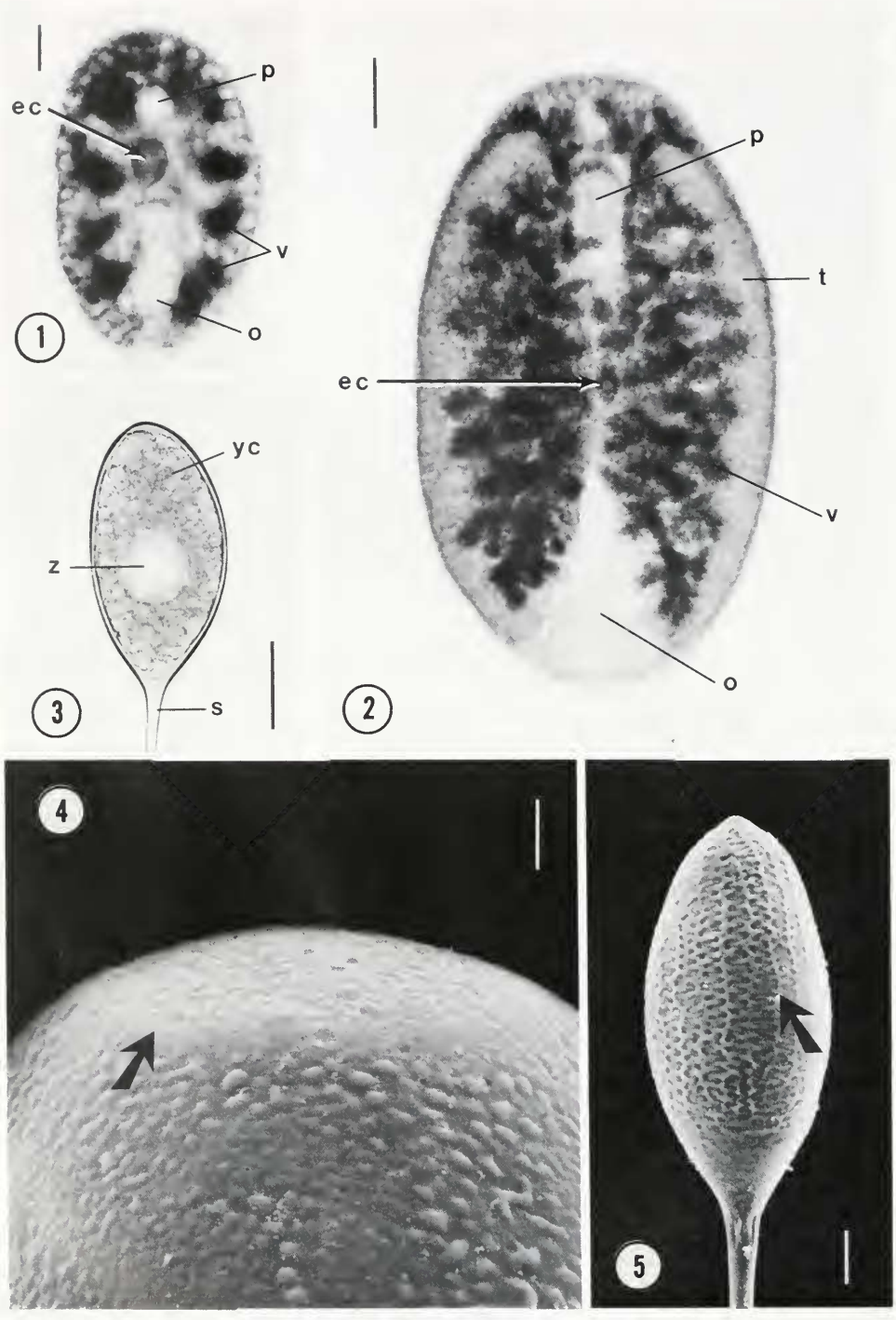

FIGURE 1. Small reproductive specimen (live) of *Anoplodium hymanae;* dorsal view. Photomicrograph; ec, egg capsule; o, ovary; p, pharynx; v, vitellaria. Scale = 100 μm.

FIGURE 2. Large reproductive specimen (live) of *A. hymanae;* ventral view. The egg capsule (ec) is larger in absolute size but much smaller relative to the size of the parent than the egg capsule of the specimen in Figure 1. Photomicrograph; o, ovary; p, pharynx; t, testis; v, vitellaria. Scale = 250 μm.

digested in papain (Adolph's Meat Tenderizer) and the digestate was examined for egg capsules. Before treatment, the body walls were washed thoroughly with fresh water. The body walls were then sliced into pieces and placed in a large beaker along with about 50 ml of fresh water and approximately 100 gm of the enzyme preparation. Digestion was carried out at room temperature for 48 h. Control egg capsules that had been dissected out of sea cucumbers were treated in a papain solution of the same concentration for the same duration.

Electron microscopy

For scanning electron microscopy, egg capsules deposited by worms kept in sea water, and brown bodies collected from hosts, were fixed in phosphate-buffered OsO_4 (Cloney and Florey, 1968). The capsules were punctured with a razor blade soon after immersion in the primary fixative; if not punctured, the capsules collapsed during dehydration. The capsules were dehydrated in an ethanol series, transferred to 2,2-dimethoxypropane, critical point dried from CO_2, coated with Au/Pd, and examined with a JEOL JSM-35 scanning electron microscope (SEM).

Results

Anoplodium hymanae is hermaphroditic. It becomes reproductive when it reaches a length of about 0.5 mm, and it grows to a maximum length of about 2.5 mm (Figs. 1, 2). Mature worms never contain more than a single egg capsule; these capsules always contain very young embryos. Thus, egg capsules are produced and released into the coelom one at a time, in continuous succession.

Morphology of egg capsules

Egg capsules of *Anoplodium hymanae* consist of an oblong bulb that is drawn out at one end into a hollow filamentous stalk. Newly produced capsules contain a single zygote (rarely 2–3 zygotes) and several dozen yolk cells (Fig. 3). The egg capsules measure 75–200 μm long by 45–97 μm wide. They vary in size with the size of the parent. Zygotes and yolk cells are of fairly constant dimensions (36–40 μm diam. and 15–18 μm diam., respectively). Capsules produced by large worms contain many more yolk cells than capsules produced by small worms. Small capsules are smooth or decorated by small verrucae (Fig. 4); capsules longer than about 160 μm have an elaborate pattern or ridges on the outside (Fig. 5). The anterior end of the bulb bears an opercular suture (Figs. 4, 10). Both the bulb and stalk are formed from secretions of the yolk cells. In some other species of umagillids, the "stalk" is a separate structure formed of secretions of the so-called cement glands (*e.g., Syndesmis echinorum,* Meixner, 1923; *Syndisyrinx franciscanus,* Shinn, 1983b). *Anoplodium hymanae* lacks cement glands (Shinn, 1983a).

FIGURE 3. Egg capsule of *A. hymanae* released *in vitro.* The capsule has a bulbous part that contains a zygote (z) and numerous yolk cells (yc); the stalk (s) of the capsule is continuous with the wall of the bulbous part. Scale = 50 μm.

FIGURE 4. Scanning electron micrograph of distal ¼ of medium-sized egg capsule of *A. hymanae;* capsules of this size are covered by small bumps. The position of the opercular suture is indicated by an arrow. Scale = 10 μm.

FIGURE 5. SEM of bulb of large egg capsule of *A. hymanae.* The bulbous part of the capsule is decorated by a network of longitudinal ridges with interdigitating side branches (arrow). Scale = 25 μm.

As noted in the original description of *Anoplodium hymanae*, specimens in a single host are often of two conspicuously different sizes (Shinn, 1983a). Egg capsules recovered from these hosts are commonly of two distinct sizes.

Rate of egg capsule production and duration of embryogenesis

Small reproductive specimens of *Anoplodium hymanae* that were kept in sea water produced and released egg capsules at a rate of about one per day (Table I). Large worms produced an average of about nine normal capsules a day, though some specimens produced considerably more (Table I). All large worms produced some abnormal egg capsules. The latter were usually narrower than normal capsules and were incomplete at the anterior end so that they would not hold eggs or yolk cells. Abnormal capsules are sometimes found in the coelom of *Stichopus californicus* but usually not in so high a proportion to normal capsules as was produced *in vitro*. The abortive capsules produced *in vitro* might have been normal if the worms had been in their hosts. Including the abnormal capsules, large worms produced an average of about 13 capsules per day.

Embryos in egg capsules that were deposited *in vitro* completed development in 30–35 days (see *Morphology of larvae* section in Shinn, 1985). Fully developed larvae were typically quiescent but were capable of ciliary and muscular movement. The embryos survived in the capsules for over ten months after development was completed. The embryos eventually died without hatching.

Status of egg capsules in the coelom

Egg capsules of *Anoplodium hymanae* occur either singly in the perivisceral coelomic fluid of the host or, more commonly, grouped within spherical masses of host coelomocytes (Figs. 6, 7). The masses of coelomic cells vary from a fraction of a mm to over one mm in diameter, and contain 25–300 egg capsules in sea cucumbers that are moderately infested. Most of the coelomocytes composing the masses are irregular in shape and contain numerous yellow-brown inclusions that give the masses an orange to brown color. The masses of coelomocytes are referred to as "brown bodies" (Hyman, 1955; Hetzel, 1965). Some brown bodies may be free in the coelom; they come out when the host is sliced open. Others adhere to the peritoneum lining the body wall. Brown bodies with egg capsules are particularly abundant among the suspensors that connect the rectum to the body wall.

TABLE I

Production of egg capsules by Anoplodium hymanae *kept in sea water*

	Small worms (0.7–1.0 mm long)		Large worms (2.0+ mm long)	
	Mean (n = 16 worms)	Range for individual worms	Mean (n = 16 worms)	Range for individual worms
Number of normal capsules released in 48 h	1.6	0–3	18.6	12–32
Number of normal plus abnormal capsules released in 48 h	1.6	0–3	26.6	16–38

Most of the egg capsules that occur singly in the coelom contain young embryos that have not begun to incorporate yolk cells. Egg capsules within brown bodies contain embryos in various stages of development, including many that have completed development (Figs. 8, 9). In a sample of 1500 capsules recovered from the coelomic brown bodies of six hosts, 1443 (96%) contained live embryos, 28 (2%) contained dead and disintegrating embryos (Fig. 10), and 25 (2%) had the operculum dislodged and lacked an embryo. Embryos in 4 (0.3%) capsules were in the process of hatching. Most of the capsules containing dead embryos and all of the capsules that were open or hatching were of the size produced by small worms. A cursory examination of egg capsules in brown bodies of other hosts (more than 70 sea cucumbers) revealed that no hosts contained more than a dozen opened capsules.

Host reaction to egg capsules

Some of the single egg capsules in the coelom are naked but most single capsules and all capsules in brown bodies are individually ensheathed by one or more layers of flattened coelomocytes (Fig. 11). The ensheathing cells usually lack the yellow-brown inclusions that are characteristic of other coelomocytes in brown bodies. In general, the ensheathed capsules remain intact and the embryos continue to develop. Coelomocytes were present, however, inside the bulb of the few open and empty capsules that were present in brown bodies.

The individual ensheathment of egg capsules of *Anoplodium hymanae* appears to constitute a specific host reaction to the capsules, but formation of brown bodies does not occur solely in response to the presence of egg capsules of *A. hymanae*. Brown bodies are present in uninfested specimens of *Stichopus californicus*, and some brown bodies in infested specimens are devoid of egg capsules. Brown bodies in the polian vesicles of parasitized and unparasitized sea cucumbers do not contain egg capsules. Some brown bodies consist entirely of host coelomocytes. Others contain calcareous ossicles of host origin, gamontocysts of gregarine protozoans, or occasionally, diatom frustules or dead copepods, in addition to, or instead of, egg capsules of *A. hymanae*.

Effects of evisceration

Two specimens of *Stichopus californicus* were observed eviscerating in aquaria on the day after they had been collected (31 Aug. 1982). Specimens of *Anoplodium hymanae*, as well as brown bodies with egg capsules, were expelled along with the hosts' intestine and respiratory trees (Table II). In both cases, evisceration was far from effective in eliminating all egg capsules or worms from the host. Of the 75+ sea cucumbers dissected during this study (see Shinn, 1985), only one had eviscerated in the field before it was collected (18 Nov. 1981); it contained 7 worms and 98 egg capsules.

Release of infective stages without evisceration

Egg capsules of *Anoplodium hymanae* were expelled in large numbers at frequent intervals from all of eight *Stichopus californicus* that were kept in individual screen-bottomed aquaria (Table III). All of the sea cucumbers had fully differentiated viscera when dissected at the end of the experiment. Nearly all of the expelled egg capsules were enclosed within brown bodies.

The brown bodies passed from the hosts were about 1 mm in diameter. They contained between 8 and 385 (mean 81) capsules. The average number of capsules released per day from individual hosts varied from 6 to 214. Egg capsules were released from four *Stichopus californicus* that contained no reproductive worms (Table III).

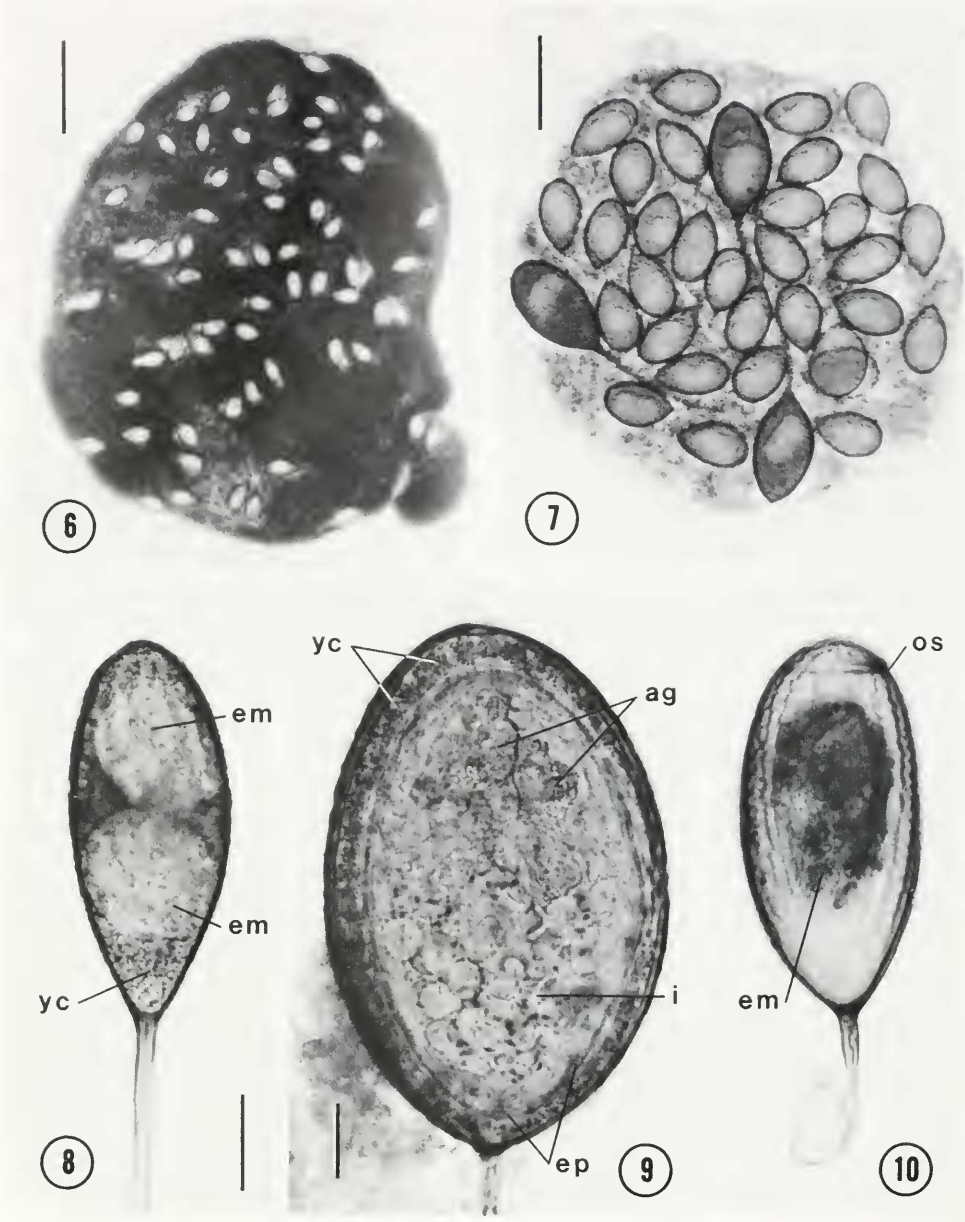

FIGURE 6. Mass of coelomocytes (*i.e.*, a "brown body") recovered from perivisceral coelom of *Stichopus californicus*, containing at least 65 egg capsules of *Anoplodium hymanae;* the volume of host cells filling the areas between egg capsules is large; the egg capsules are all large in size; specimen flattened considerably. Scale = 500 μm.

FIGURE 7. Brown body recovered from the coelom of *S. californicus* containing egg capsules of *A. hymanae* which are of two distinctly different sizes; the large capsules are similar in size to the capsules in the brown body of Figure 6; the volume of host cells filling the areas between egg capsules is small. Scale = 150 μm.

FIGURE 8. Egg capsule of *A. hymanae* removed from a brown body of *S. californicus;* the capsule contains two incompletely developed embryos (em); there are no differentiated organs and the embryos are immobile. The embryos are surrounded by yolk cells (yc) which have not yet been incorporated. This capsule is unusual because most capsules of *A. hymanae* contain only one embryo. Scale = 50 μm.

Similarly, large capsules were released from one host that contained no large worms (Table III). The worms that had deposited the capsules were no longer present in the hosts. Dissections of freshly collected animals have revealed that reproductive worms are also lost in nature (see Shinn, 1985). Rates of capsule release were calculated from data for hosts D and E (Table III) because reproductive worms had not obviously been lost from them. Large capsules were expelled at rates of 31.3 and 9.25 per day per large reproductive worm in the coelom of hosts D and E, respectively; small capsules were expelled at rates of 1.0 and 1.6 per day per small reproductive worm.

In addition to the typical coelomic brown bodies, hundreds of small (most less than 0.2 mm in diameter), dark purple or black masses of host cells that were visibly distinct from the coelomic brown bodies were recovered from the aquaria. These smaller masses never contained egg capsules of *Anoplodium hymanae*. Cells in the smaller masses contained inclusions similar to those in the cells composing coelomic brown bodies. No subadult or mature specimens of *A. hymanae* were recovered outside of the hosts. Fecal strings that did not fall through the mesh were removed when they were noticed and examined with a dissecting microscope. No egg capsules of *A. hymanae* were found in them, although egg capsules of an undescribed species of *Ozametra* that inhabits the intestine of *Stichopus californicus* were frequently found stuck to the mucus that ensheathed the feces.

Pathway of expulsion of brown bodies

The wall of the rectum of *Stichopus californicus* was found to be perforated by a ring of ducts that connect the posterior end of the perivisceral coelom to the lumen of the rectum (Figs. 12, 13). Large specimens typically have about 75 ducts but the number differs between specimens of different size. Thirteen ducts were counted in sections of a 1.5 cm long *S. californicus*. The ducts are located just internal to the anal sphincter and pass straight through the wall of the rectum. They were observed in live, anesthetized specimens by pulling open the anal sphincter and examining the wall of the rectum with the aid of a dissecting microscope. Coelomic brown bodies containing egg capsules and irregularly shaped masses of coelomocytes containing methylene blue that had been injected into the holothuroids could be seen, through the translucent rectum wall, among the suspensors of the rectum. The brown bodies and masses containing methylene blue were easily forced out of the coelom, through the ducts, and out the anus, by applying pressure to the body wall of the sea cucumber. The histological relationship of the ducts to the surrounding tissues will be described in a separate paper (Shinn and Stricker, in prep). No pores or ducts that either directly or indirectly connect the perivisceral coelom to the outside were found in other parts of the body of *S. californicus*.

Neither egg capsules nor large masses of cells identical to coelomic brown bodies were observed within the solid tissues of specimens of *Stichopus californicus*. The body wall, intestine, rectum, and respiratory trees of *S. californicus* typically contain hundreds of small, dark accumulations of cells resembling the small dark masses of cells recovered

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FIGURE 9. Egg capsule of *A. hymanae* containing a fully developed embryo; capsule compressed slightly. The anterior glands (ag), epidermis (e), and intestine (i) are visible; the embryo is capable of ciliary and muscular movement. The egg capsule was removed from a brown body from the perivisceral coelom of *S. californicus*. Scale = 25 μm.

FIGURE 10. Egg capsule of *A. hymanae* containing a dead, disintegrated embryo (em). The opercular suture (os) is conspicuous; the operculum is easily dislodged. The capsule was removed from a brown body in the coelom of *S. californicus*. Scale = 50 μm.

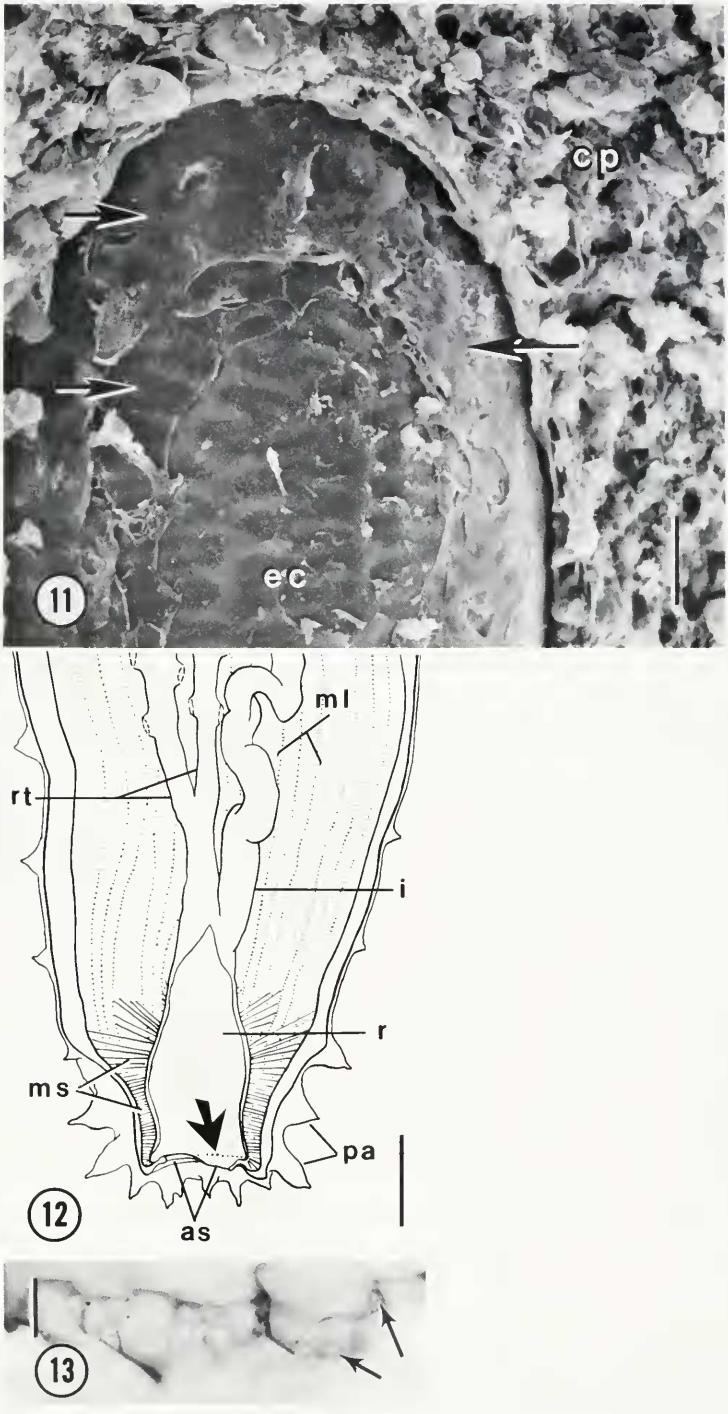

FIGURE 11. SEM of egg capsule of *Anoplodium hymanae* in a brown body of *Stichopus californicus*. The egg capsule (ec) is ensheathed by flattened host coelomocytes (arrows); the pattern of ridges on the surface of the egg capsule is visible where the sheath of coelomocytes has been removed; cp, coelomocyte packing. Scale = 10 μm.

TABLE II

Numbers of egg capsules and Anoplodium hymanae *expelled at evisceration* versus *numbers remaining in the coelom after evisceration of* Stichopus californicus

	Expelled with viscera		Retained in coelom after evisceration	
Host	Egg capsules	Worms	Egg capsules	Worms
A	221 (8.4%)	1	2405 (91.6%)	19
B	300+	33	300+	10

from the bottom of the aquaria in the capsule-expulsion experiments that were mentioned previously. Of several hundred of these masses that were teased apart or that were examined in squashes of tissues from each of five highly infected sea cucumbers, none contained egg capsules. No egg capsules of *Anoplodium hymanae* were found among the papain-digested remains of the body walls of two *S. californicus* that harbored more than 100 *A. hymanae* each. Control egg capsules were not digested by papain.

Status of embryos in expelled egg capsules

Most capsules expelled by intact hosts contained embryos in an advanced stage of development, or embryos that had completed development (Table IV). Twelve (0.6%) of the 2094 capsules examined were open and empty (Table IV). Although quantitative results were not recorded for capsules expelled during evisceration nearly all of those capsules contained viable embryos in various stages of development.

DISCUSSION

Escape of infective stages

Egg capsules of *Anoplodium hymanae* are deposited into the coelom of the host, *Stichopus californicus,* soon after they are formed. The capsules get ensheathed by small numbers of coelomocytes and, subsequently, accumulated with additional coelomocytes into the large masses that constitute brown bodies. The vast majority of capsules are transported out of the host before hatching, as was hypothesized for *A. parasita* by Schneider (1858). In contrast to previous hypotheses (Changeux, 1961; Jespersen and Lützen, 1971), the capsules are most commonly externalized without evisceration of the host. The existence of permanent ducts between the coelom and the rectal lumen, the accumulation of brown bodies with egg capsules in the vicinity of the ducts, and the fact that brown bodies containing egg capsules can be forced

FIGURE 12. Line drawing of dissection of posterior end of *S. californicus.* The body wall was sliced open and folded back; the rectum (r) was opened to reveal the openings of the coelomic ducts (arrow) into the lumen of the rectum. The rectum is attached to the body wall by the radiating suspensor muscles (ms); as, anal sphincter; i, intestine; ml, longitudinal muscles of body wall; pa, papillae on outside of body wall; rt, respiratory trees. Scale = 2 cm.

FIGURE 13. Photomicrograph of openings of coelomic ducts (arrows) into the lumen of rectum. Specimen prepared as described for Figure 12, then fixed in formalin to make the tissues opaque. Scale = 0.5 mm.

TABLE III

Numbers of egg capsules of Anoplodium hymanae *released from intact* Stichopus californicus

Host	Dates of experiment	No. of egg capsules recovered				No. of reproductive worms in coelom		
		From aquarium			From coelom			
		Total	Lg.*	Sm.	Total	Total	Lg.	Sm.
A.	3–12 Oct. 81	139 (1)**	nd	nd	76 nd	4	0	4
B.	3–12 Oct. 81	110 (10)	110	0	58 nd	0	0	0
C.	1–8 Dec. 81	90 (10)	1	89	146 nd	24	0	24
D.	6–26 Aug. 82	3327 (19)	3127	200	3888 (44)	15	5	10
E.	6–26 Aug. 82	741 (25)	185	556	300+ (36)	18	1	17
F.	5–25 Sept. 82	107 (4)	nd	nd	472 (40+)	0***	0	0
G.	5–25 Sept. 82	290 (7)	nd	nd	2 (29+)	0***	0	0
H.	5–25 Sept. 82	4071 (41)	nd	nd	79 (55)	0	0	0

* See *Intensity and seasonality of infestation* in Shinn (1985) for detailed explanation of size classes of worms and capsules.

** Numbers in parentheses represent the numbers of brown bodies that contained the capsules.

*** Small, non-reproductive worms were recovered from these hosts

nd = No data.

through the ducts by squeezing anesthetized hosts, strongly suggest that brown bodies with egg capsules are normally expelled through the ducts.

Except for the lesion that forms at the anterior end of the rectum during evisceration, neither permanent nor temporary openings that either directly or indirectly connect the perivisceral coelom to the outside have been described for aspidochirote holothuroids. Coelomic pores have been described for two species of apodan holothuroids (Becher, 1912; Anderson, 1966), however, and pores that form as temporary breaks in the posterior end of the rectum have been described for the molpadonian holothuroid *Caudina chilensis* (Kawamato, 1927; Kitao, 1935). The discovery of coelomic ducts or pores in rather cryptic locations in widely divergent species suggests that such openings may be common in holothuroids. Echinoids may also have cryptic coelomic ducts. Eight species of umagillids are reported to inhabit the intestine and coelom of echinoids (reviewed by Shinn, 1984), and presumably deposit egg capsules into the perivisceral coelom of their hosts. If these reports are valid (see Shinn, 1981), there must be a mechanism by which the egg capsules exit the coelom.

Stichopus californicus has four types of coelomocytes: amoebocytes, morula cells, lymphocytes, and crystal cells (Hetzel, 1963). Hetzel (1965) determined that, of these cell types, only amoebocytes are actively involved in phagocytic activity and the formation of brown bodies in three species of dendrochirote holothuroids and one species

TABLE IV

Developmental stage of embryos in egg capsules of Anoplodium hymanae *released from intact* Stichopus californicus

Date	Host*	Total no. capsules examined	Total no. open capsules	Percent of capsules containing embryos of specific stages**			
				1 Zygote	2 Mid-development	3 Developed	4 Dead
1–8 Dec. 1981	C.	90	0	0	61	20	19
6–26 Aug. 1982	D.	993	3	0	31	69	0
6–26 Aug. 1982	E.	497	9	10	48	42	0
5–24 Sept. 1982	G.	50	0	2	18	78	2
5–24 Sept. 1982	H.	464	0	0	6	94	0
Total		2094	12	3% (n = 11)	37% (n = 146)	55% (n = 215)	5% (n = 18)

* Host symbols correspond to those in Table III.

** Percentage based when possible on a subsample of 100 capsules.

1. Embryos the size of zygotes, epidermal layer not visible (Fig. 3).
2. Medium-sized embryos with a visible epidermal layer but without differentiated internal organs (Fig. 8).
3. Fully differentiated embryos, capable of movement (Fig. 9).
4. Embryos disintegrating (Fig. 10).

of apodan holothuroid. In some echinoids, morula (= spherule) cells as well as amoebocytes are involved in encapsulation of foreign material (reviewed by Smith, 1981). Further study is needed to verify that amoebocytes alone are involved in the ensheathment of the egg capsules of *Anoplodium hymanae* and their accumulation into brown bodies.

Brown bodies are formed by holothuroids, echinoids, and, to a lesser extent, ophiuroids (Smith, 1981; Jangoux, 1982). Structures that have the same name, but that are not homologous, have been reported for asteroids (Johnson and Beeson, 1966; Smith, 1981). Coelomocytes in brown bodies are commonly thought to be degenerate. It has been hypothesized that the yellow-brown inclusions that characterize the cells consist of the metabolic remains of pigments derived from the food (reviewed by Jangoux, 1982), and that brown bodies are formed as a means of sequestering the unwanted materials. The origin, composition, and functions of the inclusions are, however, unknown. Inclusion of foreign materials that have been ensheathed by coelomocytes is presumably an additional function of brown bodies, and is obviously associated with the defense mechanisms of the host.

Many authors have reported that individual coelomocytes, brown bodies, or other masses of coelomocytes containing foreign materials that have been injected into the coelom, can migrate out of echinoderms. In holothuroids this has been reported to involve virtually all tissues of the body (intestine, body wall, respiratory trees, etc.;

Hetzel, 1965), although in members of other classes of echinoderms it may involve only localized parts of the body (reviewed by Endean, 1966). I found no evidence that coelomic brown bodies with egg capsules migrate through the tissues of the body wall or wall of the intestine or respiratory trees of *Stichopus californicus*. My observations were fairly limited and do not disprove the latter possibility, but reveal that expulsion of egg capsules by that process is at least uncommon. The small dark masses of cells in the solid tissues of *S. californicus* and those recovered from the aquarium in my capsule releasing experiments are, by definition, brown bodies. They do not contain egg capsules of *Anoplodium hymanae*, which suggests that they do not originate in the perivisceral coelom. Only brown bodies in the perivisceral coelom contain egg capsules. This observation reflects the fact that *A. hymanae* occurs only in the perivisceral coelom, and it also demonstrates that the host is compartmentalized with regard to the movement of brown bodies that originate in different parts of the body.

Some egg capsules of *Anoplodium hymanae* are released to the sea during evisceration. It is doubtful, however, that the number of capsules expelled from eviscerating holothuroids is increased above the number that would be released in the absence of evisceration. Results of my experiments reveal that adult worms, which are also expelled during evisceration, are unable to invade new hosts. Worms expelled during evisceration may be able to reinfect the holothuroid from which they came because the gut of the holothuroid would be open to the coelom. But unless reinfection of the old host occurs regularly, evisceration probably has a net negative effect on the worm populations.

The coelom of aspidochirote holothuroids is inhabited by gregarine protozoans and greatly modified gastropod molluscs belonging to the family Entoconchidae as well as by umagillid turbellarians (Barel and Kramers, 1977). Except for those species of entoconchids that maintain an open connection to the gut lumen or outside (reviewed by Lützen, 1979), evisceration has, until now, been the only known mechanism for release of infective stages of these parasites. The involvement of coelomic ducts in escape of infective stages of these groups of parasites from the coelom should be investigated in detail.

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